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Survey of Gallibacterium anatis and Its Antimicrobial Susceptibility Pattern in Village Chickens (Gallus gallus domesticus) in Maiduguri, North-eastern Nigeria

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Abstract

The aim of this study is to determine the prevalence of Gallibacterium anatis; isolate the bacterium and determine its antimicrobial susceptibility pattern in apparently healthy village chickens in the study area. Out of the total of 150 samples which comprises of 75 tracheal and 75 cloacal swab samples collected from apparently healthy chickens of both sexes and various age groups analysed, 37 samples were found positive for Gallibacterium anatis with an overall prevalence rate of 24.67%. The bacterium was more prevalent in village chickens sampled from village poultry farmers households (17.33%) compared to those sampled from the live birds markets (7.33%). Isolation of the bacterium was more frequent in the tracheal swabs (18.67%) than in cloacal swabs (6.0%) (P = 0.0055). The prevalence of the bacterium was higher in the females (24.67%) than in the male (0.0%) chickens (P<0.0001 at 95% CI) and was also more prevalent in the adult than the young chickens (P<0.0001 at 95% CI). The prevalence of the bacterium among village chickens in this study may be associated with inadequate husbandry systems and poor hygiene. The non-haemolytic strain of the bacterium was more prevalent among the isolates compared to the haemolytic strains. The isolate of G. anatis showed negative reactions to urease, coagulase, indole and maltose test, but showed positive reactions to test with catalase, sucrose, phosphatase oxidase and sorbitol test. The antibiotic susceptibility pattern revealed that isolates were highly susceptible to ciprofloxacin and gentamycin, moderately susceptible to streptomycin and ofloxacin but resistant to amoxicillin, ceftriaxone and chloramphenicol. To control the spread of the bacterium among poultry species, adequate biosecurity measures should be put in place in all level of village poultry production system and initiation of public awareness against misuse of antibiotic by poultry farmers to avoid drug resistance.

Keywords

Gallibacterium anatis; Antimicrobial susceptibility pattern; Village chickens (Gallus domesticus); Maiduguri; North-Eastern Nigeria

Introduction

Village poultry production is the livestock enterprise available to all farming families, even the poorest ones in most developing countries of the world [1-3]. These consist of the edible domestic birds that include chickens, ducks, Guinea fowls, geese, pigeons and turkeys [4].

Many developing nations have made spectacular achievements in the area of poultry production during the last three decades but this has not improved the availability of poultry products and purchasing power of rural masses [5-7]. Unfortunately, poultry production is faced by a number of constraints which include predators, poor housing and management, inadequate feeding and most importantly, the menace of infectious diseases which have been identified as one of the major constraints to successful village chicken production [8-11]. The trend of high losses of village poultry due to diseases poses a serious threat to food security and livelihood of many rural poultry farmers. Most of the disease causing agents in poultry industries has been reported to be transported from one poultry flock to another through contaminated equipment, vehicles, servicemen as well as infected birds [12].

Gallibacterium anatis belongs to the *Pasteurellaceae* family and is a common bacterium found in the upper respiratory tract and lower reproductive tract of healthy chickens and turkeys [13-17]. Several researchers have associated *G. anatis* to pathological conditions, such as salpingitis, peritonitis, septicemia, pericarditis, hepatitis, enteritis, and respiratory tract lesions [18]. Most recently, Neubauer et al. [19] found that *G. anatis* was an agent that was very frequently associated with peritonitis and/or salpingitis in free-range laying hens showing clinical symptoms of the above mentioned lesions.

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Gallibacterium anatis is a Gram-negative, rod shaped, non-motile, capsulated, facultative anaerobic bacteria classified in the family *Pasteurellaceae* that is commonly associated with poultry [16,20] and it was recently classified as an emerging cause of disease of domesticated and domiciled birds [21]. *Gallibacterium anatis* has recently been recognized as a major cause of lesions in the reproductive tracts of egg layers [19,22,23], causing a drop in egg production and increased mortality.

Gallibacterium anatis has two biovars i.e., a haemolytic biovar, Haemolytica and a non-haemolytic biovar, anatis [24]. Currently, G. anatis and G. Genomospecies 3 and Gallibacterium group V are the defined members of the genus Gallibacterium [25]. Though the infection of G. anatis is treatable with antibiotics, the frequency of treatment failure is an emerging and recurrent problem [21]. Multiple-drug resistance [24] and a substantial antigenic diversity [26] make it difficult to prevent the negative effects of G. anatis using traditional antimicrobial agents and vaccines.

Multidrug resistant strains of *G. anatis* have shown resistance to sulphamide drugs, Novobiocin, Tylosin, Clindamycin, Tetracycline and Penicillin [24]. Concerns have been shown for bio-security measures towards control of the disease, handling of pathogen and prevention of spread. The organisms have been reported in domesticated poultry species in some part of the world [13,27,28] including Nigeria [18,29]. This present study aimed to determine the prevalence of *Gallibacterium anatis* in apparently healthy village chickens and its antimicrobial susceptibility pattern in Maiduguri, Borno State, Nigeria.

Materials and Methods

Study area

Maiduguri, also called Yerwa by its locals, is the capital and largest city of Borno State and it is located within the Sahel savannah zone of the Northeastern Nigeria. The city sits along the seasonal Ngadda River which disappears into the Firki swamps in the areas around Lake Chad. It lies approximately between 11° 5' and 11.83° N latitude and 13° 09' and 13.50° E longitude at about 350m (1161 ft) above sea level with ambient temperatures of 40-45°C (http://www. unimaid.edu.ng/About_Maid.aspx). The climate is hot and dry for a greater part of the year with a rainy season from June to September in the Northern part and May to October in the Southern part with a mean annual rainfall and temperature of about 650 mm and 32°C, respectively. The mean relative humidity ranges from 30% to 50% with the minimum usually experienced in the months of February and March, when it drops to as low as 10% and reaches maximum as high as 90% in August. Maiduguri is estimated to have a population of 1,907,600, as of 2007.

Study population

The birds sampled for the study are apparently healthy village chickens of both sexes and various age groups reared in village chicken farmer households under extensive management system and village chickens brought for sales or dressing at live bird markets within the study area. The chickens sampled were considered apparently healthy, because they were not showing any signs of illness such as diarrhea, depression, drowsiness and respiratory signs at the time of sampling.

Study design

The present study was designed based on a convenience non-probability sampling method to aseptically collect swab samples from apparently healthy village chickens from the study locations within the study area. Consent for sample collection was sought from chickens' owners. Each selected chicken was gently grabbed by the shanks for a proper manual restrain. Researchers ensure human handling of chickens during sample collections to prevent unnecessary stress and struggle.

Study/Sampling location

The study locations mapped out for the study were chosen based on the described large population of village chickens flocks and for the convenience of sample collection and safe transportation of samples collected. The study locations include village chickens farmers' households located at Gwange ward; Fori ward and the University of Maiduguri Staff quarters. Sample were also collected from village chickens brought for sales and or dressing at the live birds markets located at the Monday market and Custom markets in the study area.

Period of sample collection

Swab samples were aseptically collected from apparently healthy village chickens from the months of November, 2016 to February, 2017. Sampling locations were visited at alternate days throughout the sampling period following the consent of the village chicken owners within the study locations.

Sample collection

A total of 150 swab samples were aseptically collected from 75 apparently healthy village chickens following standard procedures for swab sample collections using sterile swab sticks. The swab samples which include tracheal and cloaca swabs were collected from each selected village chickens of both sexes and various age groups from village chickens farmers households and live birds markets at different times and different sample areas. Swab samples were appropriately labeled and transported in cold boxes immediately to the University of Maiduguri, Department of Veterinary Medicine Diagnostic and Research Laboratory for processing.

Media Preparation

The media used for the study are Nutrient agar and MacConkey agar were prepared according to standard laboratory procedures adopting the protocol previously described by Tankeshwar et al. [30] but with slight modification.

Microbiological isolation of Gallibacterium anatis

Streak plate method to obtain pure cultures was the microbiology method employed for isolation of the bacterium. The procedure was carried out according to the standard microbiological procedure previously described [30].

Cultivation for microscopy examination

Petri dishes were inoculated with swab samples and incubated at room temperature for 24-48 hours in an incubator following guided stages of standard procedures for microbiological samples cultivation as previously described [31,25]. Standard procedures were adopted for the making of thin smear of the pure cultures on glass slides for the microscopy examination.

Grams staining procedure used for the study

Grams staining procedure used for the study was carried out according to the guidelines of standard procedures for Gram's staining. Observation of isolates motility was carried out according to standard procedures previously described by [16].

Biochemical Test

The following biochemical tests which include Oxidase test, Phosphatase test, Nitrate Reduction test, Catalase test, Indole test, Urease test, were performed to confirm the reaction of *Gallibacterium anatis* isolates in the study. The tests were carried out according to standard procedures previously described [30, 16].

Antimicrobial susceptibility test

Antimicrobial sensitivity tests can guide the physician in drug choice and dosage for difficult to treat infections. Results are commonly reported as the Minimal Inhibitory Concentration (MIC), which is the lowest concentration of dug that inhibits the growth of the organism. A pure colony of the *G. anatis* isolate was picked using a sterile wire loop, then placed into a normal saline in a bottle, the bottle was shaken gently to ensure homogenous spread

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of the organism and then pour into a Petri dish containing agar, the drug" antibiotic sensitivity disc" is placed firmly into the Petri dish and incubated. Antimicrobial susceptibility testing of *G. anatis* isolated was performed using disc diffusion test. The antimicrobials used include Cefotaxime, Florfenicol, Norfloxacin, Ciprofloxacin, Gentamycin, Erythromycin, Ampicillin, Amoxicillin, Cephradine, Doxycycline, Oxytetracycline, Sulphamethoxazole + Trimethoprim, Streptomycin, Lincomycin, and Spectinomycin. All isolates were cultured overnight on 5% citrated sheep blood agar at 37°C in microaerophilic condition, then cultures were suspended in 0.85% NaCl to an optical density equivalent to that of McFarland 0.5 standards. Each isolate was then inoculated onto Mueller Hinton agar medium, then 15 minutes later, the antimicrobial discs were applied. Plates were incubated anaerobically at 37°C for 24 hours and the interpretation was done according to the manufacturer description.

Statistical analysis

The G. anatis contamination status of any chicken was recorded based on microbiological isolation of the bacterium from the tracheal and cloacal swabs collected. A chicken was regarded contaminated when the tracheal, the cloacal or both samples from the individual chicken were positive for G. anatis test. A flock was classified as positive if just one chicken was recorded positive in that flock. The data obtained from the study were analysed using prevalence (%) and 95% confidence interval to determine the differences which was evaluated by Chi square test and statistical significance between variables at P < 0.05 was considered significant.

Results

The diagnosis of *Gallibacterium anatis* in swab samples collected from adult and young village chickens (*Gallus domesticus*) of both sexes from households that rears large population of local breeds of poultry species and from live birds markets in Maiduguri was based on the phenotypic characteristics exhibited by the colonies on blood agar plates and their biochemical reactions. A total of one hundred and fifty (150) swab samples were collected which comprise of 75 tracheal swabs and 75 cloacal swabs from apparently healthy village chickens. Out of the total samples collected, *G. anatis* was isolated and identified in pure culture from 37/150 samples with an overall prevalent rate of 24.67% as shown in Table 1.

The isolation of Gallibacterium anatis from swab samples collected from village chickens based on location of sample collections revealed high prevalence rate of the bacterium in sample collected from households 26/74 (35.14%) compared to samples collected from live birds markets 11/76 (14.47%) with prevalent rates of 17.33% and 7.33%, respectively. The result of *G. anatis* isolation from samples collected from live birds markets shows higher prevalence of the bacterium isolate in samples collected from Monday market 8/40 (20.0%) compared to Customs market 3/36 (8.33%) at a prevalence rate of 5.33% and 2.0%, respectively. However, considering samples collected from households, swab samples collected from village chickens in Gwange ward 20/30 (66.67%) had the highest frequency of *G. anatis* followed by village chickens from Fori ward 6/30 (20.0%), the bacterium was not isolated from swab samples collected from village chicken of University of Maiduguri staff quarters 0/14 (0.0%) as shown in Table 2.

The prevalence of *Gallibacterium anatis* in village chickens (*Gallus domesticus*) according to the type of samples collected revealed higher frequency of the bacterium in the tracheal swab 28/75 (37.33%) compared to the cloacal swab 9/75 (12.0%) at a prevalence rate of 18.67% and 6.0%, respectively. Moreover, there is statistical significant difference (P<0.01) (Table 3).

Number of samples collected	Number of samples contaminated (%)	Prevalence (%)
150	37 (24.67)	24.67

Table 1: Prevalence of *Gallibacterium anatis* in village chickens (*Gallus domesticus*) in Maiduguri, Borno State, Nigeria

The prevalence of *G. anatis* isolated from village chickens according to age and sex shows higher prevalence of the bacterium in the adult chickens 37/102 (36.27%) sampled compared to the young 0/48 (0.0%) at a prevalence rate of 24.67% and 0.0%, respectively (P<0.0001). The result of the isolation of *G. anatis* from both sexes of village chickens revealed higher prevalent rate of the bacterium in the female chickens 37/88 (42.02%) compared to the male ones 0/62 (0.0%) at a prevalence rate of 24.67% and 0.0% respectively, (P<0.0001) (Table 4).

The result of distribution of *Gallibacterium anatis* isolated from village chickens in pure culture based on their haemolytic and non-haemolytic characteristics of isolate on blood agar revealed that the non-haemolytic strain 26/37 (70.27%) of the bacterium is more frequently isolated than the haemolytic strain 11/37(29.73%). Isolation of the non-haemolytic strain was more frequent in cloacal swabs 9/9 (100.0%) compared to the tracheal swabs 17/28 (60.71%) while the haemolytic strain of the bacterium was only isolated from the tracheal swab 11/28 (39.29%) as shown in Table 5.

The result of the biochemical test for *G. anatis* isolated in pure culture from village chickens revealed that the 37 positive samples shows positive reactions to test with catalase, oxidase, phosphatase, sucrose, and sorbitol, however demonstrate negative reactions to indole, urease, coagulase and maltose as shown in Table 6.

The result of the antimicrobial sensitivity test shows that *Gallibacterium anatis* has strong susceptibility to Ciprofloxacin, Streptomycin, Gentamycin, and ofloxacin compared to Pefloxacin, Cefuroxime and Nitrofurantion, and high resistance to Ceftriaxone, Amoxicillin and Chloramphenicol as shown in Table 7.

Discussion

Gallibacterium anatis infection is an emerging disease of poultry. Growing concern about G. anatis is its poorly understood growth kinetics, virulence markers, pathogenesis and vaccine(s) to control [21]. Bojesen et al. [13] have previously mentioned that G. anatis is a normal flora of both the upper respiratory tract and lower genital tract of many avian species. The present study relied on diagnosis of Gallibacterium anatis infection based on the phenotypic characteristics of the isolated organism on blood agar. The isolation of the bacterium in 24.67% of the sampled chickens in this study indicated the presence of the organism among scavenging village chicken flocks in the study area. This finding is lower than 96% reported by Bojesen et al. [13] in free range local breed of chickens. This bacterium have also been previously isolated from other breeds of chickens as well as a wide range of other domestic birds including turkeys, geese, ducks, pheasants, partridges and cattle egrets [16,20,23,27,31-33]. In most instances high prevalence of the infection were recorded in scavenging chickens usually reared in an environment with poor bio security. From the result of the present study, the prevalence of G. anatis was higher in scavenging village chickens sampled at households 74 (86.67%) compared to those sampled from the live birds markets 76 (28.33%). The major reasons responsible for these differences in the prevalent rates may be associated with free ranging and poor management systems which usually predispose scavenging chickens to high chance of ingestion of various pathogens including *G. anatis* in a contaminated environment and getting infections compared to the movement restricted chickens in live birds markets. These finding buttress previous report of [13] and [34] who have also reported high prevalent rate of the bacterium in free range chickens than chickens reared in a secured environment and have attributed it to poor bio-security. Moreover, the isolation of G. anatis in apparently healthy village chickens from live birds markets may not be unexpected, because the chickens are in most instances sourced directly from poultry farmers households and when they gets to the live birds markets, there are usually no discriminations of health status or screening for diseases before mixing chickens from different sources. Although, the present study did not analyze how the prevalence of *Gallibacterium* was related to the breed or ecotype of the chickens. The result of our study also showed a significantly higher proportion of tracheal swab samples positive for G. anatis

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Sample location Specific Area		No. of sample collected (N 150)	No. of sample contaminated (%)	Prevalence %	
	Monday Market	40	8 (20.0)	5.33	
Live birds market	Custom Market	36	3 (8.33)	2.0	
	Total		11 (14.47)	7.33	
	Gwange Ward	30	20 (66.67)	13.33	
Household	Unimaid Staff Quarters	14	0 (0.0)	0.0	
	Fori Ward	30	6 (20.0)	4.00	
Total		74	26 (35.14)	17.33	

Keyword: N = Total number of samples collected; Unimaid= University of Maiduguri

Table 2: Prevalence of *Gallibacterium anatis* in village chickens (*Gallus domesticus*) according to sampling locations in Maiduguri Borno State, Nigeria

Type of samples collected	No. of sample collected	No. of sample affected (%)	Prevalence (%)	95% CI LL – UL	<i>P</i> -value	RR
Tracheal swab	75	28 (37.33)	18.67	0.6311 - 0.8111		
Cloacal swab	75	9 (12.0)	6.0	0.8064 - 0.9498	0.0055	0.8155
Total	150	37 (24.67)	24.67			

Table 3: Prevalence of Gallibacterium anatis in village chickens (Gallus domesticus) according to type of samples collected in Maiduguri Borno State, Nigeria

than the corresponding cloacal swab samples from the same infected village chicken in a flock. It is suggestive that the tracheal followed by the cloaca region might be the more favourable predilection site of the bacterium. This might be associated with the affinity of the bacterium for the upper respiratory system compared to the lower genital tract. This finding agrees with previous studies that reported natural cases of the bacterium mainly targeting the upper respiratory and lower reproductive tract of birds [13,19,27,28,35-37].

However, the result of our study showed that the proportion of *G. anatis* positive samples independent of the two anatomical site of isolation to be significantly high since this is the first time the bacterium was isolated from chickens in Northeastern Nigeria. Therefore, this makes it very probable that the bacterium could be detected in either the trachea or cloaca location when examining just a few chickens from an infected flock as suggested by Bojesen et al. [13].

The results of this study indicated that adult female village chickens could be more susceptible to G. anatis infection with no isolate found in the young females or in the adult and young male (P < 0.0001 95% CI). Our finding buttress the previous report of Bojesen et al. [13] who reported no positive results in young chickens and suggested that vertical transmission does not seem to be a typical mode of transfer of infection for members of the Gallibacterium. The unlikelihood of vertical transmission leaves major efforts of eradication to be centered on minimizing horizontal transmission of the pathogen by means of maintaining strict bio security measures. Therefore, taking into consideration the route and mode of transmission of the bacterium among susceptible chickens flocks is an important guiding factor in the perspective of designing an eradication strategy.

The occurrence of the bacterium in female village chickens might coincidently have a negative effect on the female reproductive organs and in its egg production physiology. Although, detecting the pathological changes of the reproductive organs in the female infected chickens was not within the scope of the present study. However,

previous findings of [25,27,34] have reported evidence association of the bacterium to pathological changes in the female reproductive organs of infected chickens. Moreover, [36] have also investigated the pathogenesis of *G. anatis* in free range male chickens and have reported the bacterium in the reproductive tract of cockerels. This evidence indicated that the occurrence of the bacterium in chickens' reproductive organs might have negative impact and as well on the long-run retard the productivity of the infected bird.

The finding of our study have indicated that *G. anatis* isolated from apparently healthy village chickens demonstrated negative reactions to indole, urease, coagulase and maltose and positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol. This finding is in line with those of [17] and [38] who have also reported similar biochemical reactions of *G. anatis* isolates. The results of this study revealed that the non-haemolytic strain of Gallibacterium was predominantly isolated from pure culture of the positive samples compared to the haemolytic strain. This indicated that the nonhaemolytic G. anatis is the most naturally abundant strain of the bacterium among household village chickens in the study area and infected chickens might have gotten infection during scavenging on contaminated environment. This finding supported previous report of [16,38] and [28] who have also reported the susceptibility of scavenging chickens to G. anatis infection from contaminated environment. Although, [13] in a similar study have reported prevalent of Gallibacterium species in Danish chicken production systems that were characterized as having low to moderate bio security levels, indicating that lesser bio security is a major risk factor for obtaining a Gallibacterium infection.

The *in vitro* susceptibility pattern of *G. anatis* isolates to different antimicrobials compounds in the present study indicated that isolates of the bacterium from village chickens were highly susceptible to Ciprofloxacin and Gentamycin, moderately susceptible to Streptomycin, Ofloxacin as well as fairly susceptible to Nitrofurantion, Cefuroxime and Pefloxacin, but were completely resistant to

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Risk factors	Variable	No. of sample collected	No. of sample affected	Prevalence (%)	95% CI LL – UL	P -value	RR
	Young	48	0 (0.0)	0.0	-		
Age	Adult	102	37 (36.27)	24.67	0.6519 - 0.8047	P < 0.0001	1.363
	Male	62	0 (0.0)	0.00	-		
Sex	Female	88	37 (42.05)	24.67	0.6164 - 0.7825	P < 0.0001	1.420

Key: CI = Confidence interval; RR= Relative risk

Table 4: Prevalence of Gallibacterium anatis according to age and sex of village chicken in Maiduguri Borno State, Nigeria

Type of sample collected	No. of infected samples tested (n = 37)	Type of Gallibacterium biovar isolated		
		Non-haemolytic (%)	Haemolytic (%)	
Tracheal swabs	28	17 (60.71)	11 (39.29)	
Cloacal swabs	9	9 (100.0)	0 (0.0)	
Total	37	26 (70.27)	11 (29.73)	

Table 5: Haemolytic characteristics of isolated serovars of Gallibacterium anatis from village chickens on blood agar

Biochemical test	No of sample tested (N =37)	
Urease	-ve	
Indole	-ve	
Catalase	+ve	
Coagulase	-ve	
Maltose	-ve	
Sucrose	+ve	
Sorbitol	+ve	
Phosphatase	+ve	
Oxidase	+ve	

Key: +ve = Positive test; -ve = Negative test

Table 6: Biochemical identification for Gallibacterium anatis isolated from village chicken in Maiduguri Borno State, Nigeria

Erythromycin, Cephradin, Oxytetracycline, Sulpha. + Trimethoprim, Amoxicillin, Chloramphenicol and Ampicillin. The result of our study supported previous reports of antimicrobial susceptibility patterns and resistance of *G. anatis* reported by several researchers. [24] Have reported resistance of field strains G. anatis isolates to Tetracycline and Sulfamethoxazole-Trimethoprime. Bojesen et al. [38] and Guo [39] have also reported that all G. anatis isolates were susceptible to Amoxicillin-Clavulanic acid, Apramycin, Cefpodoxime, Ceftifur, Cephalotin, Chloramphenicol, Colistin, Florfenicol, Gentamycin, Spectinomycin, Streptomycin, Erythromycin, Tiamulin and Tilmicosin but their isolates were resistant to tetracycline, Sulfamethoxazole, Ciprofloxacin and Nalidixic acid. While, El-Bestawy [40] reported a complete resistance of G. anatis isolates against 5 different antimicrobials; Oxytetracycline, Sulphamethoxazol + Trimethoprim, Lincomycin and Spectinomycin and the isolates were sensitive to Chloramphenicol.

Conclusion

Gallibacterium anatis is prevalent among the village chickens reared in the study area, with the non-haemolytic strain being the predominant strain of the bacterium which was more frequently isolated from the trachea compared to the cloaca samples tested. The occurrence of the bacterium in swabs samples collected from apparently healthy village chickens is attributed to normal floral

or natural infection by horizontal transmission probably from contaminated environment since there was no previous report of serious outbreak of disease caused by the bacterium in the study area. The unhygienic scavenging nature of village chickens might be considered as the most predisposing factor of the diseases transmission among free range village chickens. Also the indiscriminate mixing of several poultry species in live birds local markets might also contribute to the horizontal transmission of the organism. The *in vitro* antimicrobial susceptibility of the isolated bacterium has demonstrated multidrug resistance, but susceptible to a few ones, this suggested that the bacterium can be treated with some antimicrobial chemotherapy.

Recommendations

Gallibacterium anatis is an emerging poultry disease; it is therefore recommended that poultry farmers should be educated about the economy significance of the disease. The isolation of *G. anatis* should be attempted in most uncertain clinical disease of female chickens such as drop in egg productivity both in sizes and numbers. The indiscriminate use of antibiotic and antimicrobial compounds in village chicken and other poultry production systems should be strictly discouraged. To control disease transmission to susceptible birds, strict bio security measures should be observed in all levels of poultry production systems.

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Antibiotics	Degree of Antimicrobial susceptibility of isolates				
· intubiotics					
Ciprofloxacin (10MCG)	+++				
Gentamycin (10MCG)	+++				
Streptomycin (30MCG)	+++				
Cefuroxime (10MCG)			+		
Pefloxacin (10MCG)		++			
Ceftrixone (30MCG)				RR	
Amoxicillin (30MCG)				RR	
Ofloxacin (10MCG)					
Chloramphenicol (10MCG)		++		RR	
Nitrofurantion (100MCG)			+		

Key: +++ = Highly susceptible; ++ = Moderately susceptible; + = Fairly susceptible; RR = Completely resistant

Table 7: Antimicrobial susceptibility of Gallibacterium anatis isolated from village chickens in Maiduguri, Borno State, Nigeria

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Competing Interests

The authors declare that they have no competing interests.

References

- Alders RG (2004) Poultry for profit and pleasure.FAO Diversification Booklet 3. Food and Agriculture Organization of the United Nation: Rome
- Alexander DJ, Bell JG, Alders RG (2004) Technology review: Newcastle disease with special emphasis on its effect on village chickens. FAO Animal production and Health Paper No.161, Rome FAO63n
- 3. Bagnol B. Improving village chicken production by employing effective gender sensitive methodologies. In: Village Chickens, Poverty Alleviation and the Sustainable Control of Newcastle disease. Proceedings of an international conference; (2005) Oct 5–7; Dar es Salaam, Tanzania.
- Njue SW, Kasiiti JL, Macharia JM, Garcheru SG, Mbugua HCW. Health management improvements of family poultry production in Africa: survey results from Kenya. In: Characteristics and Parameters of Family Poultry Production in Africa. Publication of FOA / IAEA Co – ordinated Research Programme; (2002). 39 – 45.
- Copland JW, Alders RG (2005) The Australian village poultry development Programme in Asia and Africa. World's Poultry Science Journal 61: 31–37.
- Nwanta JA, Abdu PA, Ezema WS (2008) Epidemiology, challenges and prospects for Control of Newcastle disease in village poultry in Nigeria. Poultry Science Journal 64: 119-127.
- 7. Echeonwu BC, Ngele MB, Echeonwu GON, Joannis TM, Onovoh EM, et al. (2008) Response of chickens to oral vaccination with Newcastle disease virus vaccine strain I_2 coated on maize offal. Afr J Biotechnol 7: 1594–1599.
- 8. El-Yuguda AD, Dokas UM, Baba SS (2005) The effects of Newcastle disease and Infectious bursal disease vaccines, climate and other factors on village chicken population in North eastern Nigeria. Journal of Food, Agriculture and Environment 3: 55–57.
- 9. El-Yuguda AD, Ngulde IS, Abubakar MB, Baba SS (2007) Village chicken health, management and production indices in selected villages of Borno state, Nigeria. Family Poultry 17: 41–48.
- 10. Musa U, Abdu PA, Dafwang II, Edache JA, Ahmed MS, et al. (2008). A survey of causes of mortality in some Local chicken flocks in Plateau state: In: Proceedings of the 33rd Annual Conference of

- the Nigeria Society of Animal Production (NSAP), ACIAR,pp. 551 554.
- Geidam YA, KA, Grema HA Rabana JL, Sanda (2011) A Survey of Health and Management Problems Associated with Rural Poultry Production in Gombe Metropolis North-eastern Nigeria. Sahel Journal of Veterinary Sciences 10: 77–81.
- 12. Lister SA (2009). Bio-security in Poultry Management. Poultry Disease Elsevier's Health Service Right Dept., 6th edition: pp. 50 56.
- 13. Bojesen AM, Nielsen SS, Bisgaard M (2003a) Prevalence and transmission of haemolytic *Gallibacterium* species in chicken production systems with different bio security levels. Avian Pathology 32: 503–510.
- Bojesen AM, Torpdahl M, Christensen H, Olsen JE, Bisgaard M (2003b) Genetic diversity of *Gallibacterium* anatis isolates from different chicken flocks. jcm 41: 2737–2740.
- Bojesen AM, Christensen H, Nielsen OL, Olsen JE, Bisgaard M (2003c) Detection of *Gallibacterium spp*. in chickens by fluorescent 16S rRNA in situ hybridization. J Clin Microbiol 41: 5167–5172.
- 16. Christensen H, Foster G, Christensen JP, Pennycott T, Olsen JE, et al. (2003a) Phylogenetic analysis by 16S rDNA gene sequence comparison of avian taxa of Bisgaard and characterization and description of two new taxa of Pasteurellaceae. J Appl Microbiol 95: 354–363.
- 17. Christensen H, Bisgaard M, Bojesen AM, Mutters R. Olsen JE (2003b) Genetic relationships among avian isolates classified as *Pasteurella haemolytica, Actinobaccilus salpingitidiso, Pasteurella anatis* with the proposal of *Gallibacterium anatis* gen. nov., comb. Nov. and description of additional genomospecies within Gallibacteriumgen. nov. Int J Syst Evol Microbiol 53: 275-87.
- 18. Addo PB, Mohan K (1985) Atypical *Pasteurella haemolytica* type A from poultry. Avian Diseases 29: 214 217.
- 19. Neubauer C, De Souza-Pilz M, Bojesen AM, Bisgaard M, Hess M (2009) Tissue distribution of haemolytic *Gallibacterium anatis* isolates in laying birds with reproductive disorders. Avian Pathology 38: 1–7.
- 20. Bisgaard M, Korczak BM, Busse HJ, Kuhnert P, Bojesen AM, et al. (2009) Classification of the taxon 2 and taxon 3 complex of Bisgaard within Gallibacterium and description of *Gallibacterium* melopsittaci sp. nov., *Gallibacterium trehalosifermentans* sp. nov. and *Gallibacterium salpingitidis* sp. nov. Int J Syst Evol Microbiol. 59: 735-44.
- 21. Singh SV, Singh BR, Sinha DK, Kumar VOR, Vadhana PA, et al. (2016) J Veterinar Sci Techno 7: 324.
- 22. Jordan FTW, Williams NJ, Wattret A, Jones T (2005) Observations on salpingitis, peritonitis, and salpingoperitonitis in a layer breeder flock. Vet Rec 157: 573-577.

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- Jones KH, Thornton J K, Zhang Y, Mauel M J (2013) A 5-year retrospective report of *Gallibacterium anatis* and *Pasteurella multocida* isolates from chickens in Mississippi. Poult Sci 92: 3166-3171.
- 24. Bojesen AM, Vazquez ME, Bager RJ, Ifrah D, Gonzalez C, et al. (2011) Antimicrobial susceptibility and tetracycline resistance determinant genotyping of Gallibacterium anatis. Vet Microbiol 24: 105-10.
- 25. Bojesen AM, Christensen JP, Bisgaard M (2008) *Gallibacterium* infections and other avian Pasteurellaceae, p 160–163. In Pattison M, McMullin PF, Bradbury JM, Alexander DJ (ed), Poultry diseases, 6th ed. Saunders/ Elsevier, Edinburgh, United Kingdom.
- 26. Vazquez ME, Gonzalez C De, la, Mora, R. and Bojesen AM (2006) Prevalence of *Gallibacterium anatis* in Mexico and their effect in laying hens, poster 25-29/6 –2006. 4th International Veterinary and Vaccines Diagnostics Conference, Oslo, Norway.
- 27. Sorour HK, Al Atfeehy MN, Azhar G, Shalaby AG (2015) Gallibacterium anatis Infection in Chickens and Ducks. Assiut Veterinary Medical Journal 61: 80–86.
- 28. Abd El-Hamid H S, Ellakany H F, Bekheet A A, Elbestawy AR, Mataried N (2016) Pathogenicity of ten *Gallibacterium anatis* isolates in commercial broiler chickens. Alexandria J Vet Sci 49: 42-49.
- 29. Lawal JR, Ndahi JJ, Bello AM, Wakil Y, Dauda J, et al. (2017) Prevalence, isolation and antimicrobial susceptibility pattern of *Gallibacterium anatis* from local breed of female Muscovy ducks (Cairinamoschata) in Maiduguri, Northeastern Nigeria. J Vet Med Anim Sci 2: 27–37.
- Tankeshwar A (2013) Overview of Biochemical tests for differentiating Gram positive cocci In: Biochemical tests in Microbiology, Laboratory diagnosis of Bacterial Disease.
- Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, et al. (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4: 295-305.

- 32. Rzewuska M, Karpinska E, Szeleszczuk P, Binek M (2007) Isolation of *Gallibacterium spp.* from peacocks with respiratory tract infections. Medycyna Weterynaryjan 63: 1431–1433.
- 33. Gregersen RH, Neubauer C, Christensen H, Korczak B, Bojesen AM, et al. (2010) Characterization of Pasteurellaceae-like bacteria isolated from clinically affected psittacine birds. J Appl Microbiol 108: 1235-43.
- 34. Persson G, Bojesen AM (2015) Bacterial determinants of importance in the virulence of *Gallibacterium anatis* in poultry. Vet Res 46: 57.
- 35. Paudel S, Alispahic M, Liebhart D, Hess M, Hess C (2013) Assessing pathogenicity of *Gallibacterium anatis* in a natural infection model: the respiratory and reproductive tracts of chickens are targets for bacterial colonization. Avian Pathology 42: 527–535.
- 36. Paudel S, Liebhart D, Aurich C, Hess M, Hess C (2014a) Pathogenesis of *Gallibacterium anatis* in a natural infection model fulfils Koch's postulates: 2. Epididymitis and decreased semen quality are the predominant effects in specific pathogen free cockerels. Avian Pathol 43: 529-34.
- 37. Paudel S, Liebhart D, Hess M, Hess C (2014b) Pathogenesis of *Gallibacterium anatis* in a natural infection model fulfils Koch's postulates: 1. Folliculitis and drop in egg production are the predominant effects in specific pathogen free layers. Avian Pathol 43: 443-449.
- 38. Bojesen AM, Vazquez ME, Robles F, Gonzalez C, Soriano EV, et al. (2007) Specific identification of Gallibacterium by a PCR using primers targeting the 16S rRNA and 23S rRNA genes. Vet Microbiol. 123: 262-268.
- 39. Guo LT (2011) Studies on drug resistance and resistant genes of *Gallibacterium anatis* strains isolated from chickens in different localities. Master Thesis in Vet. Med, China.
- 40. El-Bestawy AR (2014) Studies on *Gallibacterium anatis* infection in chickens.Ph.D thesis in Poultry Diseases, Alexandria University.

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